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CN 200310120030.8, filed December 1, 2003

Priority

HUVASTATIN AND ITS PREPARATION AND FORMULATION COMPRISING THE HUVASTATIN

FIELD OF THE INVENTION

The invention relates to statin compounds. More particularly, the invention relates to novel small molecule statin compounds, the preparation methods thereof and formulations comprising the huvastatin as active ingredients.

BACKGROUND OF THE INVENTION

In recent years of science development, it has been well known that high blood cholesterols, lipids, and unhealthy habits such as smoking and lack of exercise, are the main contributors for causing cardiovascular diseases. Abnormal lipid level is an important risk factor of atherosclerosis and coronary heart disease. The chance of getting cardiovascular diseases and death rate caused by the above reasons has shown steady growing in recent years. According to a survey, about 10~20% of adults suffer from high total cholesterol (TC) or high triglyceride (TG), and even nearly 10% of children suffer from hyperlipemia. Therefore, it is very urgent to prevent and treat cardiovascular diseases by developing therapeutically effective drugs that can regulate blood lipid. In recent years, several new groups of cholesterol reducing drugs have been developed, of which statins have attracted much attention.

Statin drugs, which are the most effective inhibitors for the 3-hydroxyl-3-methyl glutaryl coenzyme A (HMG-CoA) reductase, are the most important discoveries of the end of the twentieth century. Since the first statin compound, lovastatin, was marketed in 1987, the 3-hydroxyl-3-methyl glutaryl coenzyme A (HMG-CoA) reductase inhibitor drugs have achieved great success clinically and profitably.

The side chain structure of statin compounds has a similar moiety as hydroxyl methyl glutaryl coenzyme A, and it can competitively inhibit the cholesterol synthesis, thus it is powerful in reducing blood lipids for patients with hyperlipemia. It is demonstrated by a series of clinical trials, especially global scale clinical trials, that using statin drugs may reduce the chance of primary and secondary coronary heart disease as well as its mortality, which is the number one death causing disease in the world. The analysis results from a variety of research data have revealed that statins can effectively reduce the level of low-density lipoprotein cholesterol (LDL-C) and exhibit a linear positive correlation. Therefore, reducing the serum cholesterol level as greatly as possible will obtain greater clinical success. Presently, statins have entered the clinical trails to treat Alzheimer's disease and multiple sclerosis.

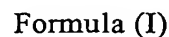
Statin inhibit the endogenous biosynthesis of cholesterol, and thus prevent the onset

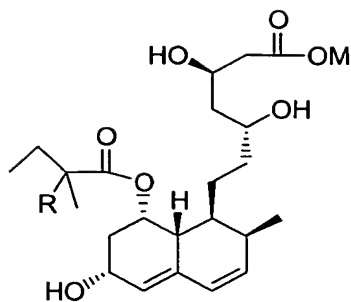
Several statins are commercially available, such as lovastatin, simvastatin, pravastatin, fluvastatin and the like. However, pravastatin exhibits higher hydrophilicity, which indicates that the passive diffusion ability of pravastatin is lower, and as a result, its dosage is comparatively high. The hydrophilicity of simvastatin is relatively low, but low solubility also lead to relatively high dosage. Moreover, a substantial percentage of patients with hyperlipidemia cannot get their blood lipid controlled at desired level by the existing statins.

The object of the invention is to provide novel small molecule statin compounds with high potency to reduce blood lipid levels, i.e., huvastatin, which can effectively prevent and treat cardiovascular diseases

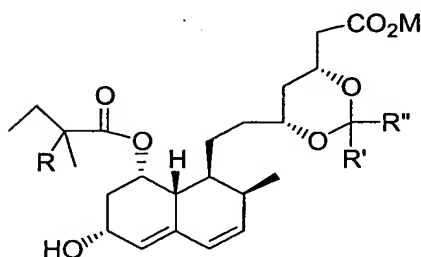
Another object of the invention is to provide the formulations comprising the huvastatin as active ingredients.

The huvastatin of the present invention, i.e., hydroxyl methyl glutaryl coenzyme A reductase inhibitor (HMG-CoA-RI) includes the first subclass, the second subclass and the third subclass, which have the following formulas respectively:





Formula (II)



Formula (III)

Wherein, R may be methyl, ethyl, propyl, *iso*-propyl, and butyl;

R' may be methyl, ethyl, propyl, *iso*-propyl, and butyl;

R'' may be methyl, ethyl, propyl, *iso*-propyl, and butyl;

M is a metal ion, such as lithium, sodium, potassium and calcium.

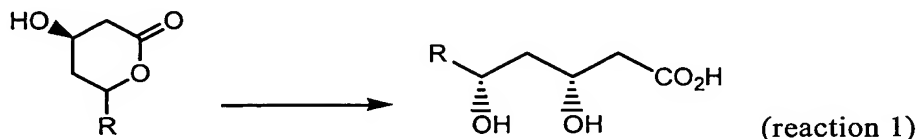
Huvastatin has the same pharmacophore dihydroxyl heptonic acid moiety as the other statin compounds, and it is a semi-synthetic small molecule statin compound starting from pravastatin. Huvastatin exhibits better therapeutic effect and lower dosage due to its suitable hydrophilicity.

For all the statins, the key bioactive moiety is the chiral β -hydroxyl heptonic acid.

Huvastatin has the same pharmacophore dihydroxyl heptonic acid moiety as the other statin compounds, but the remaining structure of huvastatin is different from the other statins. It has one more hydroxyl group than simvastatin, making it more hydrophilic. The log D of Class I huvastatin determined at pH 7.4 is 0.82, lower than most other commercially available statin compounds (about 1.1~1.7). The higher hydrophilicity of huvastatin indicates the lower passive diffusion ability, and thus it is difficult to enter non-hepatic cell. However it can be absorbed by the liver via selective organic anion transportation process and can be distributed selectively and act on HMG-CoA reductase within the liver. The relative higher solubility of huvastatin in water avoids the need of higher dosage, and avoids the extensive metabolism by cytochrome P450 before being cleared; therefore the potential drug interaction of huvastatin is expected to be lowered greatly.

Class I huvastatins of the present invention and simvastatin are both inactive lactone

compounds, which need to be hydrolyzed via liver to open the ring to become active inhibitor such as β -hydroxyl carboxylic acid, thus performing pharmacological action. The reaction is as follows:



Class II huvastatins described in the present invention are active drugs. They are active (M=H) β -hydroxyl carboxylic acids, or statin derivatives that are easily to be converted to active β -hydroxyl carboxylic acid (M=Li, Na, K, or Ca). Class II huvastatin has two more hydroxyl groups and one more carboxylic group than Class I huvastatin, resulting in a higher hydrophilicity. The log D of Class II huvastatin determined at pH 7.4 is 0.42, which is comparable to that of pravastatin, but much lower than that of most other commercially available statin compounds.

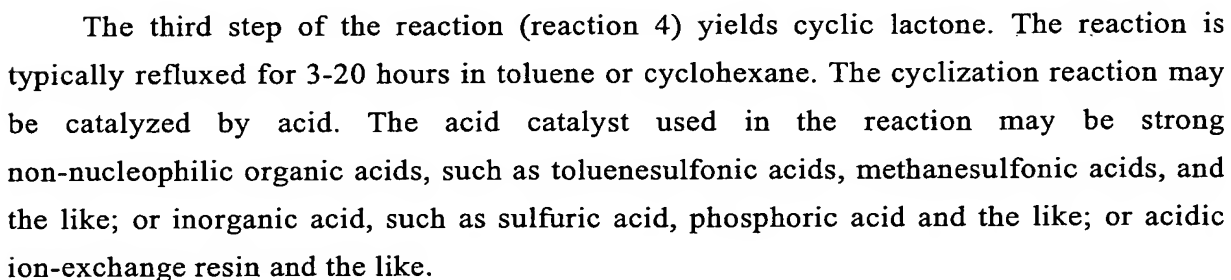
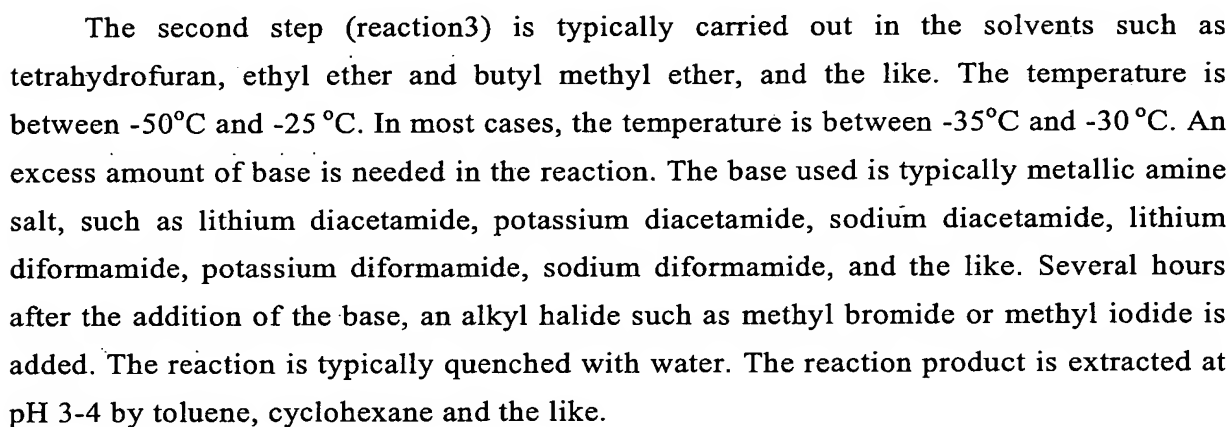
In order to facilitate the preparation of formulations and increase the chemical stability of huvastatin, the present invention devises Class III huvastatins, which are relatively more stable, and much easier to be converted into active β -hydroxyl carboxylic acid compounds. They can be converted into β -hydroxyl carboxylic acid compounds catalyzed by acid inside the stomach, which is very acidic (low pH value) environment. Due to these drugs have been converted into active inhibitors before they get to the liver, the novel statins compounds not only reduce the work load of liver, but also reduce the amount of the drugs that need to be converted in the liver, thus reducing the loss resulting from metabolism. The compounds of the present invention thus have high bioavailability and lower dosage compared with known statins.

CHEMICAL SYNTHESIS

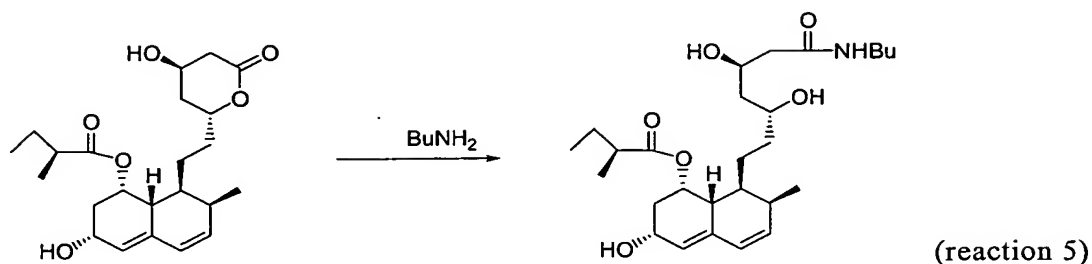
Class I huvastatin can be prepared from pravastatin. The 2-position of the 2-methylbutyryl group in the 8-position of the hydrogenated naphthalene is alkylated.

One of the representative compounds is synthesized via the three reaction steps shown below.

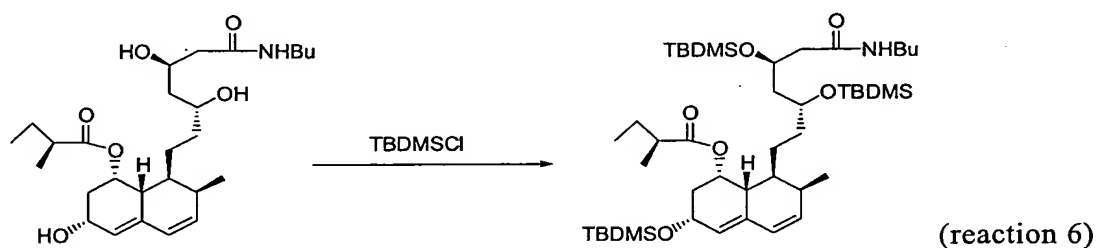
The first step (reaction 2) is a ring-opening reaction, which transforms lactone to corresponding acyclic alkali metal salt. The reaction typically uses the solvents that can form azeotropic mixture with water easily, such as benzene, toluene and cyclohexane and the like. Equivalent amount of base is used in the reaction. Preferably potassium salt, such as potassium hydroxide, is used as a base. A small amount of C1 to C3 organic alcohol is



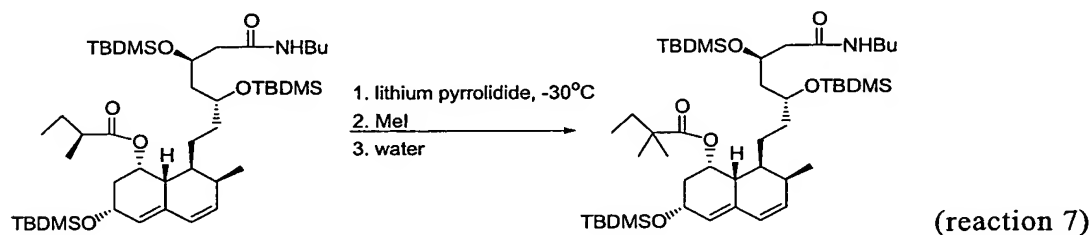
The other synthetic methods for huvastatin are shown in the following reactions 5-11:



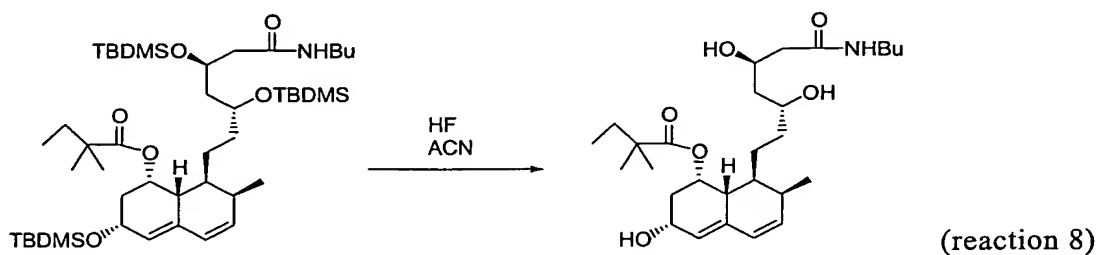
The protection of the carboxylic group: by reaction with organic the lactone ring is opened to form the corresponding amide.



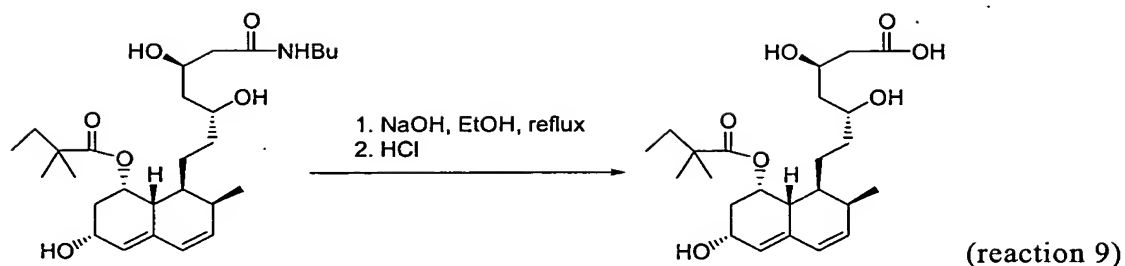
The protection of the hydroxyl group: all of the hydroxyl groups are transformed into the corresponding siloxane, such as *tert*-butyl dimethyl siloxane.



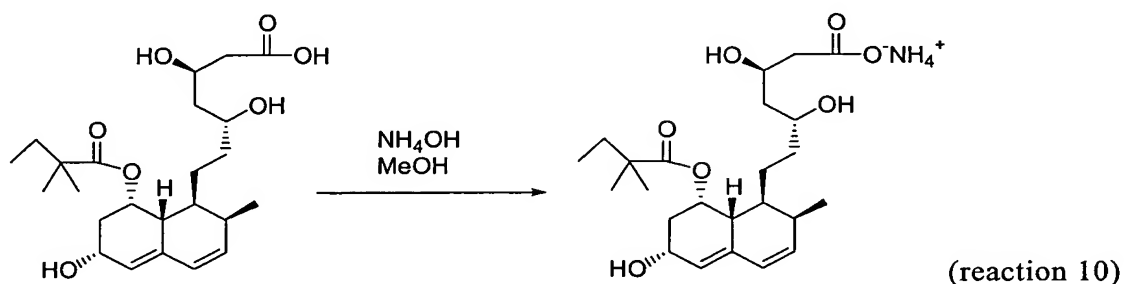
The 2-position of the 2-methylbutyryl group in the 8-position of the hydrogenated naphthalene is methylated.



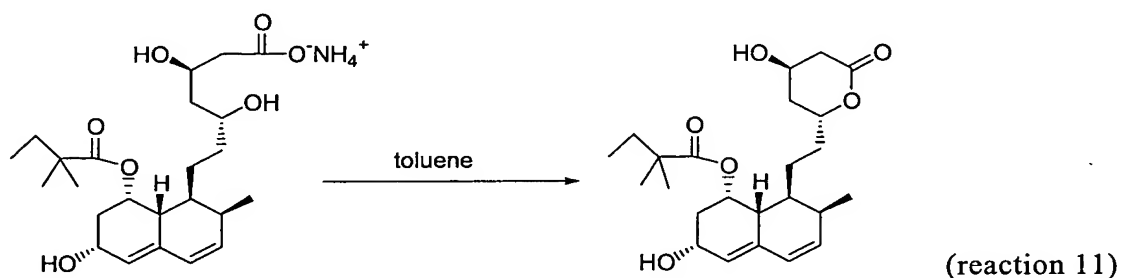
The deprotection of the hydroxy group: all the protected hydroxyl groups by the *tert*-butyl dimethylsiloxane are transformed into the corresponding hydroxyl groups.



The deprotection of the carboxylic group: the amide is transformed into corresponding carboxylic group.



The carboxylic group is transformed into the corresponding ammonium salt in order to facilitate the recrystallization and purification.

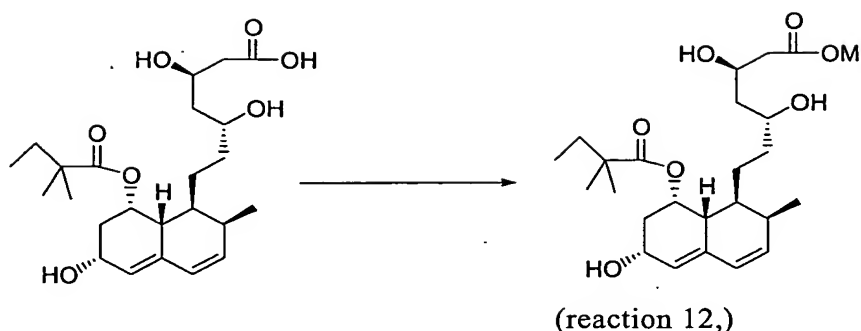


Intra-annular esterification reaction affords the corresponding Class I huvastatin, i.e., 2,2-dimethylbutyric acid-3-hydroxy-8-[2-(4-hydroxy-6-oxo-2-tetrahydro pyran-2-yl)-ethyl]-7-methyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl ester.

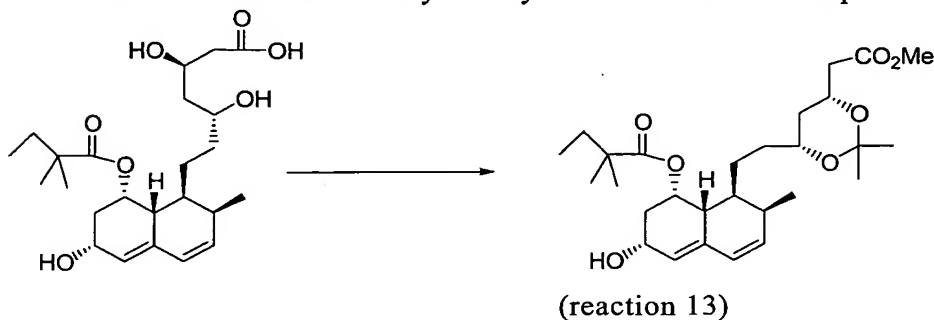
Class II huvastatin may be synthesized via one step of reaction shown below.

The salification reaction is achieved by the reaction of β -hydroxy carboxylic acid (the product of ring-opening reaction of the Class I huvastatin) with a corresponding base.

The corresponding base may be lithium hydroxide, sodium hydroxide and potassium hydroxide. The calcium salt can be obtained through cation exchange, e.g., via precipitation with calcium chloride



Class III huvastatin may be synthesized via two-step reactions shown below.



2,2-dimethylbutyric acid-3-hydroxy-8-[2-(6-methoxycarbonyl-2,2-dimethyl[1,3]-dioxanyl)-ethyl]-7-methyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl ester.

The first step is to convert the β , δ -dihydroxyl group of the starting material to 6-member ring ketal, meanwhile the carboxylic group of the molecule is converted to the corresponding ester. The reagent suitable for this step may be the corresponding ketone, or the corresponding 2, 2-dialkoxypropane. The alkoxy of the 2,2-dialkoxypropane is C_1 to C_3 alkoxy group, such as 2,2-dimethoxypropane, 2,2-diethoxypropane, 2-methoxy-2-ethoxypropane, 2,2-dipropoxypropane, and the like. The corresponding ketone may be used as the starting material, such as acetone, methyl ethyl ketone and pentanone, and the like.

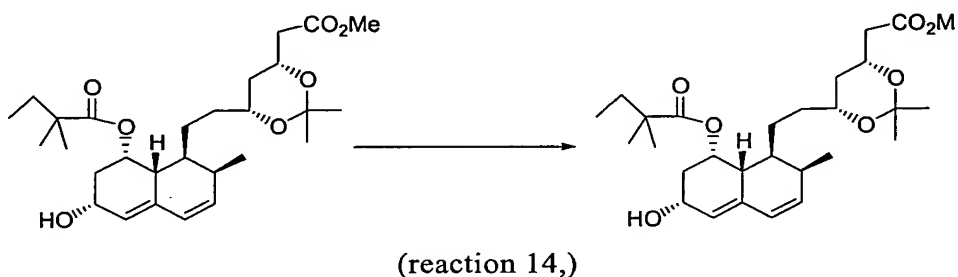
The catalyst used in the reaction may be strong non-nucleophilic organic acids, such as *p*-toluenesulfonic acids, methanesulfonic acids, and the like; or inorganic acid, such as sulfuric acid, phosphoric acid and the like; or acidic ion-exchange resin and the like.

This step of the reaction may use the corresponding reagent directly as solvent, such as 2,2-dimethoxypropane, and the like. Or the organic compounds such as toluene, dichloromethane, and dichloroethane may be used as solvent. Under the conditions, the ratio of the reagent to the starting lactone is typically between 1:1 and 2:1.

The temperature in this step is typically between -20°C to 60°C , in most cases between 0°C to 30°C . The reaction is performed under inert gas such as nitrogen. The

amount of the catalyst used in the reaction is between 0.1mol% and 100mol%, typically between 1mol% and 5mol%.

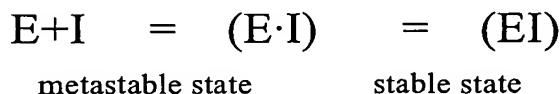
The second step is the hydrolysis catalyzed by a base. The hydrolysis reaction needs at least one equivalent of base, typically between 1 and 1.5 equivalents of base. The temperature is between -20°C to 60°C, typically between 0°C to 30°C. The solvent may be water, alcohol such as methanol and ethanol, toluene, or acetone and the like, or the combination of water and other organic solvents. When mixed solvents are used, phase transfer catalyst is needed in the reaction.



Class III huvastatin compounds are acid-labile, thus the formulation of these statin compounds need to be stabilized with necessary buffering agents. The compounds that are suitable to be used as buffering agents may be organic base, such as amino sugar and the like; inorganic base, such as sodium carbonate, sodium bicarbonate, disodium hydrogen phosphate and the like; organic salts, such as sodium acetate, and the like; metal oxide, such as magnesium oxide; amino acid, such as arginine, and the like.

The values such as inhibiting constant K_i and $\log D$ are more satisfactory compared with the other statins, showing that huvastatin is a suitable drug compound for reducing lipid levels and lowering cholesterol.

K_i is the binding (inhibiting) constant of statin compounds with 3-hydroxyl-3-methyl glutaryl coenzyme A (HMG-CoA).



The results are tabulated as below:

Name	Lovastatin	Simvastatin	Pravastatin	Atorvastatin	Huvastatin (formula II)
K_i	51	12	43	8.2	7.3

As shown in the above table, huvastatin (formula II) has smaller inhibiting constant, and its inhibiting effect is stronger than that of atorvastatin.

FORMULATIONS OF THE PREPARATION

Formulation for other statin compounds, such as capsule formulation and tablet formulation are suitable for huvastatin of the present invention. Typically, the formulation includes 1.0 to 10.0 mg active pharmaceutical ingredient and a pharmaceutically acceptable carrier or excipients. The total amount of the active pharmaceutical ingredient and lactose may be varied 0.1-100 mg.

Take tablet formulation as an example. Generally speaking, it is prepared by blending fixed ratio of the active pharmaceutical ingredient, fillers, lubricants, binding agents, disintegrants, and buffering agents, scrunching evenly (the temperature is typically controlled below 50 °C), and then pressed into tablets.

The pharmaceutically acceptable carriers or excipients described above refers to the conventional pharmaceutical carriers or excipients, for example:

The fillers may be lactose, spray dried anhydrous lactose, microcrystalline cellulose, powdered cellulose, calcium sulphate, and the like.

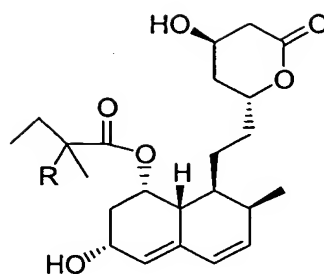
The disintegrants may be low substituted hydroxypropyl cellulose, crosslinked croscarmellose sodium, gelated silica gel, sodium hydroxymethyl starch, microcrystalline cellulose, crosslinked polyvinyl-pyrrolidone, crosslinked sodium carboxymethyl cellulose, sodium carboxymethyl starch, and the like.

The binding agents may be maltodextrin, pre-gelated starch, polyvinyl-pyrrolidone (PVP), vinyl-pyrrolidone and the copolymer of vinylacetate (KOLLIDON VA64), and the like.

The lubricants may be micro powder silica gel, magnesium stearate, or calcium stearate.

The following examples illustrate particular methods for preparing compounds in accordance with this invention. These examples are illustrative and are not to be read as limiting the scope of the invention as it is defined by the claims.

Example 1: Preparation of Class-I Huvastatin (Compound 1)



R=methyl

2,2-Dimethylbutyric acid-3-hydroxy-8-[2-(4-hydroxy-6-oxo-tetrahydropyran-2-yl)-ethyl]-7-methyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl ester

Pravastatin (12.25 mmol) was dissolved in cyclohexane (100 ml) and 2-propanol (12 ml) at room temperature under dry nitrogen, then added pre-prepared 4.91M potassium hydroxide aqueous solution (2.5 ml) into the above pravastatin solution in one portion. The reaction solution was stirred for about 0.5-1 hour at room temperature under dry nitrogen. The solid product obtained by evaporation of solvents was re-dissolved in anhydrous cyclohexane (150 ml). The solution was concentrated to a volume of 15ml, then diluted with 35 ml anhydrous THF. The prepared potassium carboxylate solution was cooled down to -35°C.

A solution of lithium pyrrolidine (3.6 ml) in anhydrous THF (30ml) was cooled to -5°C and a solution of 1.55 M n-butyllithium was gradually added in with the internal temperature maintained below 0°C during the addition. The lithium pyrrolidide was slowly added to the cooled solution of pravastatin potassium salt, maintaining the internal temperature below -30°C throughout the addition. Two hours later, a solution of methyl bromide (2.36ml) was added slowly with an internal temperature maintained below -20°C. This reaction mixture was maintained at this temperature for 1 hour.

1.55 M n-butyl lithium solution (12 ml) was added into a solution of pyrrolidine (1.6 ml) in THF (15 ml), the internal temperature maintained below 0°C. Lithium pyrrolidide solution produced in this way was gradually added to the reaction mixture maintaining an internal temperature below -30°C.

The reaction mixture was aged between -35°C and -30°C for one hour, then another portion of the methyl bromide solution (3.01g) was added slowly maintaining an internal temperature below -20°C. The reaction mixture was aged at this temperature for one hour.

The mixture was transferred into 100 ml of water. The layers were separated and the aqueous layer was adjusted to pH 4.5 with 20% phosphoric acid. The acidified aqueous phase was extracted with cyclohexane (3 X 300 ml), and combined cyclohexane extracts were washed twice with water, and then dried with anhydrous sodium sulfate.

The solution was filtered and slowly concentrated to a volume of 40 ml, then cooled

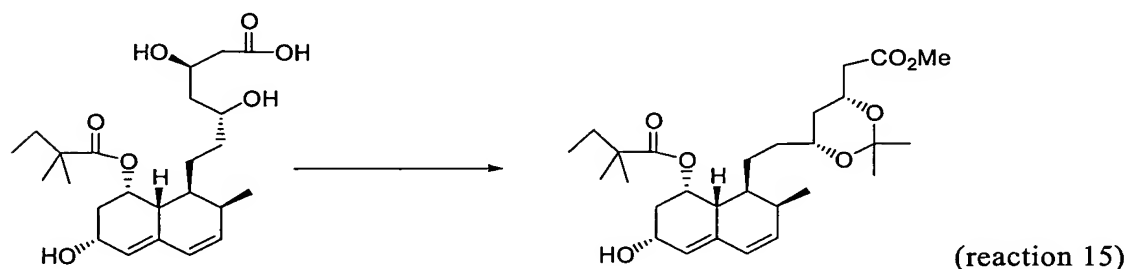
down to room temperature. The crude solid product was obtained after filtration, and the pure product of Class I Huvastatin was re-crystallized from methanol and water.

Example 2: Preparation of Class-II Huvastatin potassium salt (Compound 2)

100 mmol of the ring-opening product of Class I Huvastatin was suspended in cyclohexane (100 ml) and 2-propanol (12 ml) at room temperature under dry nitrogen, then 101 ml of 4.91 M potassium hydroxide aqueous solution was gradually added into above solution. The reaction mixture was stirred for a period of 30 to 60 minutes at room temperature under dry nitrogen. Crude solid product was obtained after distilling off solvents. The crude product was suspended in toluene and was dried through azeotropic distillation. The solid product obtained was washed with cold acetone, and then dried under vacuum to give Compound 2.

Example 3: Preparation of Class III Huvastatin (Compound 3)

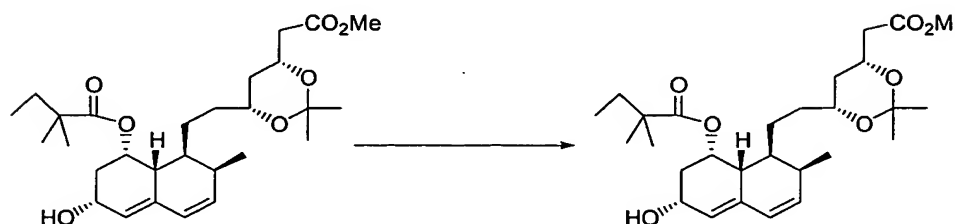
Step 1: The reaction is shown below:



100 mmol of Class-I Huvastatin carboxylic acid compound was suspended in 50 ml 2,2-dimethoxypropane at room temperature under dry nitrogen, and the mixture was stirred for more than ten minutes till all the starting materials distributed uniformly in the solution. 5 mmol of catalyst p-toluene sulphonic acid was added to this reaction mixture, and the reaction mixture was stirred at room temperature under dry nitrogen until all solids were dissolved and clear solution was obtained. After stirring at room temperature for another 30 minutes, 50 mmol of sodium carbonate was added, and kept stirring for another one hour. After filtration and rotary evaporation most of the solvents was removed under reduced pressure, the residue was separated with 200 ml ethyl acetate and 100 ml saturated sodium chloride aqueous solution. The isolated ethyl acetate phase was dried with anhydrous sodium sulfate. The 1,3-dioxane ester compound was obtained after ethyl acetate was distilled off.

Step 2: Formation of 1,3-dioxane sodium carboxylate

The reaction is shown by the equation below (M is sodium ion)



100 mmol of 1,3-dioxane ester compound was suspended in 200 ml distilled water. To this suspension, 50% sodium hydroxide aqueous solution was gradually added until homogeneous solution was obtained. The solution pH was adjusted and then maintained at 12 for one hour, after that the pH was adjusted to pH 10 with hydrochloric acid. The reaction mixture was extracted with ethyl acetate, and the ethyl acetate extract was concentrated to obtain solid crude product. The crude product was suspended in toluene and dried through azeotropic distillation. The solid product obtained was washed with cold acetone, then dried under vacuum to obtain compound 3.

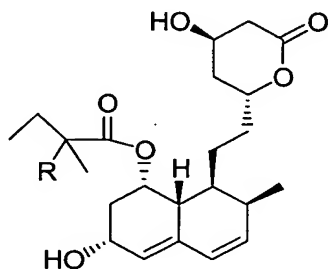
Example 4:

ingredients by weight:

Ingredients	Required amount (mg)
Active component (Compound 1, 2 or 3)	5.0
Lactose	42.0
Microcrystalline cellulose	30.0
Cellulose powder	16.0
Polyvinyl-pyrrolidone	0.5
Crosslinked Croscarmellose Sodium	5.0
Glucosamine	0.5
Magnesium stearate	1.0
Total	100

What is claimed is:

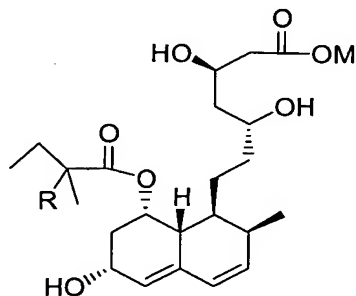
1. A novel small molecule statin compound of huvastatin, suitable for reducing blood lipid levels, with formula shown as follows:



Formula (I)

Wherein, R may be methyl, ethyl, propyl, *iso*-propyl, or butyl.

2. A novel small molecule statin compound of huvastatin, suitable for reducing blood lipid levels, which is a derivative of the compound of formula(I), with formula shown as follows:

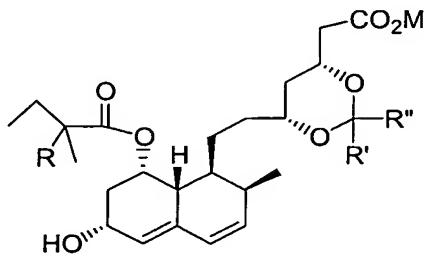


Formula (II)

Wherein, R may be methyl, ethyl, propyl, *iso*-propyl, and butyl;

M is a metal ion, such as lithium, sodium, potassium or calcium.

3. A novel small molecule statin compound of huvastatin, suitable for reducing blood lipid levels, which is a derivative of the compound of formula(I), with formula shown as follows:



Formula (III)

wherein, R may be methyl, ethyl, or propyl;

R' may be methyl, ethyl, propyl, *iso*-propyl, or butyl;

R'' may be methyl, ethyl, propyl, *iso*-propyl, or butyl;

M is lithium, sodium, potassium or calcium.

4.A synthetic method of the huvastatin of claim 1, suitable for reducing blood lipid levels, comprising the steps of:

Starting from pravastatin, after the protection of the carboxylic group with formation of alkali metal salt, the 2-position of the 2-methylbutyryl group in the 8-position of the hydrogenated naphthalene is alkylated with alkyl halide.

5.The synthetic method of the huvastatin of claim 4, wherein the alkyl halide is alkyl bromide or alkyl iodide.

6.The synthetic method of the huvastatin of claim 1, suitable for reducing blood lipid levels, comprising the steps of:

Starting from pravastatin, after the carboxylic group is converted into amide and the hydroxyl group is protected by siloxane, the 2-methylbutyryl group in the 8-position of the hydrogenated naphthalene is transformed into 2,2-dimethylbutyryl group with alkyl halide.

7.The synthetic method of the huvastatin of claim 6, suitable for reducing blood lipid levels, wherein the siloxane is *tert*-butyl dimethyl siloxane.

8. The synthetic method of the huvastatin of claim 2, suitable for reducing blood lipid levels, wherein

The β -hydroxyl carboxylic acid, i.e., the product of the ring-opening reaction of the compound of formula (I), reacts with the corresponding base to afford the compound of formula (II).

9.The synthetic method of the huvastatin of claim 8, suitable for reducing blood lipid levels, wherein the base is lithium hydroxide, sodium hydroxide or potassium hydroxide.

10. The synthetic method of the huvastatin of claim 3, suitable for reducing blood lipid levels, comprising the steps of:

In the presence of ketone or 2,2-dialkoxylpropane, the β , δ -dihydroxyl carboxylic acid, i.e., the product of the ring-opening reaction of the compound of formula (I) is converted into 6-member ring ketal by acid catalysis, then the ketal reacts with the base of formula MOH to afford the compound of formula (III).

11.The synthetic method of the huvastatin of claim 10, suitable for reducing blood lipid levels, wherein the base is lithium hydroxide, sodium hydroxide or potassium hydroxide.

12.A pharmaceutical composition comprising an effective amount of the compound of formula (I) or formula (II) or formula (III) and a pharmaceutically acceptable carriers or excipients.

Abstract

The invention relates to statin compounds, and it discloses novel small molecule compounds, i.e., huvastatins, which are classified into I, II, and III. The invention also provides the preparation methods thereof and the formulations comprising the huvastatin as
5 active ingredient. The present compounds can be used at lower dosage compared to the existing statin compounds, and also can help to control the desired blood lipid levels of the patients with hyperlipidemia. Huvastatin of the invention exhibits suitable hydrophilicity, strong potency and therapeutical effect for reducing lipid levels, and lower dosage.